inactivating lentivirus (LV). MSCs in monolayer were transduced by supplementing culture medium with LV and polybrene. Transduction efficiency was measured as % eGFP+ MSCs via flow cytometry. IL-1Ra secretion into culture media was quantified by ELISA. The chondrogenic potential of MSCs following transduction with constitutive IL-1Ra or eGFP LV was assessed in pellet culture. After 21 days in chondrogenic medium (+10 ng/mL rhTGF-β3) or a basal medium, pellets were processed for histology and stained to evaluate sulfated glycosaminoglycan (s-GAG) and collagen production as measures of chondrogenesis.

Results: Transduced MSCs constitutively produced IL-1Ra in monolayer culture for 27 days at concentrations of 3-10 ng per 10K cells every 3 days (Fig 1, A). Doxycycline-induced IL-1Ra production exceeded constitutive expression for the first 21 days. Non-transduced cells did not produce detectable levels of IL-1Ra. The magnitude of inducible IL-1Ra secretion was effectively tuned from 2-200 ng/mL with doxycycline doses ranging from 1-1000 ng/mL (Fig 1, B). IL-1Ra-transduced, eGFP-transduced (90% eGFP+), or control MSCs were differentiated in pellet culture for 21 days. Pellets in chondrogenic culture conditions produced s-GAGs far exceeding the production in basal medium, with similar levels observed in transduced and control cell populations (Fig 2, A). IL-1Ra secretion in transduced pellets ranged from approximately 100-250 ng/mL throughout the culture period (a magnitude shown to inhibit pathophysiologic IL-1 levels) (Fig 2, B). These findings show that IL-1Ra-producing MSCs can undergo chondrogenesis following transduction while simultaneously producing therapeutic levels of IL-1Ra.

Conclusions: We have developed MSCs capable of tunable IL-1Ra production at therapeutically relevant concentrations. We have shown that these MSCs retain their chondrogenic potential in pellet culture. Future studies will examine the dynamic response of MSCs in response to IL-1 challenge during chondrogenesis in pellet culture and in a tissue-engineered construct. This system shows potential to enable growth and differentiation of tissue-engineered cartilage constructs within the inflammatory environment of an OA joint. In addition to a protective effect on the engineered cartilage, release of IL-1Ra into the surrounding tissue may have therapeutic effects throughout the joint.

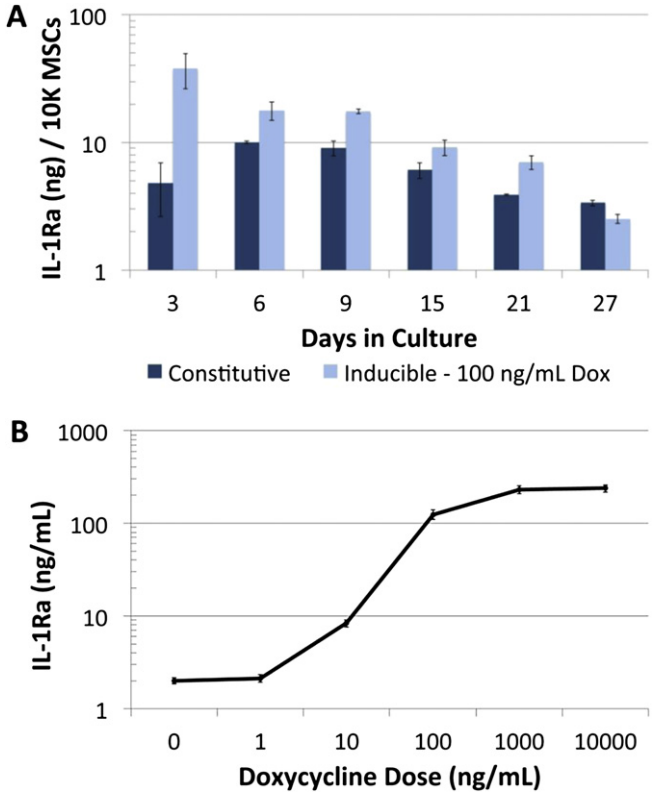

Figure 1. A. IL-1Ra secretion into media over 72 hours, from 3 to 27 days in culture (mean ± SEM, n=3). B. IL-1Ra secretion tuned by doxycycline dose. Shown is concentration released into media during 72 hours of induction (mean ± SEM, n=3).

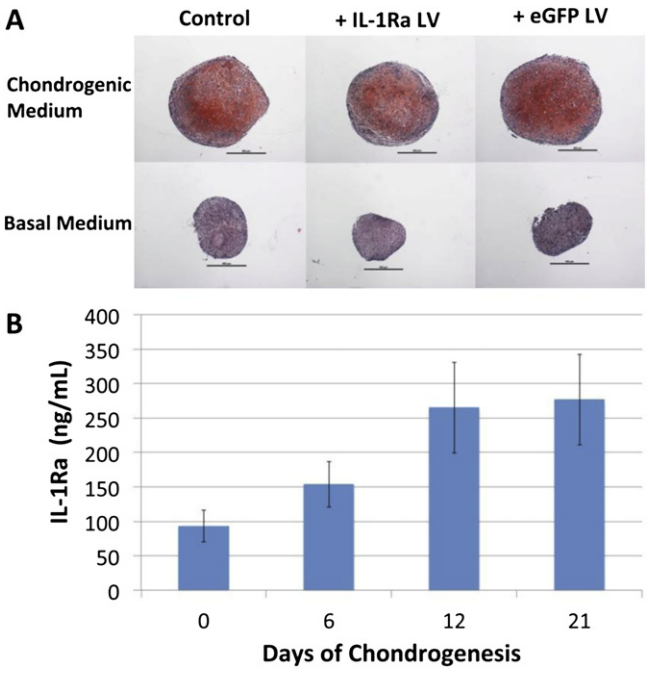

Figure 2. A. Safranin-O/fast green/haematoxylin staining for s-GAGs, collagen, and cell nuclei in MSC pellets following 21 days in culture in chondrogenic (top row) or basal medium (bottom row). 6 μm sections, scale bar = 500 μm. B. IL-1Ra secretion into media at 3 day intervals (mean ± SEM, n=3).

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CHANGES IN CHONDROGENESIS DIFFERENTIATION POTENTIAL OF BONE MARROW MESENCHYMAL STEM CELLS ACCORDING TO DONOR AGE

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Purpose: Mesenchymal stem cells (MSC) are good candidates to be used in regenerative medicine due to their differentiation potential. Different studies propose these cells for the development of osteo-articular tissues. However, while MSC from bone marrow are well characterized, recent studies have evocated a decrease in proliferation and differentiation capability of these cells when donor's age increase. The aim of this work was to study the influence of donor's age on the chondrogenic differentiation of bone marrow mesenchymal stem cells.

Methods: Two groups of donors were analyzed (<20 years and >60 years). Proliferation and clonogenicity of cells were tested for a culture period of 10 weeks. Chondrogenic differentiation of cells was induced in pellet culture using a chondrogenic differentiation medium and TGF-β3. After that, proteoglycan production was assayed using alcian blue staining and gene expression of chondrocyte markers quantified by qRT-PCR.

Results: Cells from the young donor's group an increased proliferation capacity and clonogenicity as compared with cells from old donors. In parallel, young donor group's cells were characterized by high level expression of type II collagen, aggrecan and SOX-9 genes while cells from the old donors group showed a poor chondrogenic differentiation.

Conclusions: This study shows that differences on proliferation and chondrogenic differentiation exist between mesenchymal stem cells isolated from bone marrow obtained from young or old donors. This could be considered in the choise of MSC for cartilage tissue engineering.

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EFFECTS OF CHONDROITIN SULFATE ON THE GENE EXPRESSION PROFILE IN IL-1β STIMULATED SYNOVIAL FIBROBLAST CELLS CULTURES

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